

EXHIBIT A

Purpose: Test specificity of Myco Genus Probes Mch A 1026, Mch B 1634, & Mch B 1638 against rRNA's of a few possible cross-reacting organisms.

Procedure:

- ① Set up samples & controls in 280 mM PB lysis tubes, L/N 70123
 - a) 2 - 100 μ l LNC, L/N 70224A
 - b) 2 - 100 μ l *L. Tb* rRNA, L/N 546-576-001
 - c) 2 - 1 μ g *C. xerosis* rRNA, L/N 7064 B
 - d) 2 - 1 μ g *N. asteroides* rRNA, L/N 128-503-001
 - e) 2 - 1 μ g *N. asteroides* rRNA, L/N 7025
 - f) 2 - 1 μ g *R. bronchialis* rRNA, L/N 616-572-001
- ② Add 1 ml 125 I-prbe - Mch A 1026, Mch B 1634, & Mch B 1638
~15,000 - 20,000 cpm/ml (all 125 I) by M. Hoppe
- ③ Vortex, & add incubate at 72°C for 2 hrs
- ④ Add 4 ml HA sep soln (0.12M PB, 2% HA), L/N 70147 to 10 tubes at a time on bench top.
- ⑤ Invert 80X, & incubate at 72°C for 5 min
- ⑥ Remove from bath, invert 20X, & spin at 2000 rpm for 2 min.
- ⑦ Decant & blot
- ⑧ Add 4 ml wash (0.12M PB) L/N 70145
- ⑨ Vortex 20 seconds
- ⑩ Incubate at 72°C for 5 min.
- ⑪ Spin at 2,000 rpm for 2 min, decant, & blot
- ⑫ Count for 2 min

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Read & Understood by me,

T. minor

Date

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Dr. Z. Z. Z. Z. Z.

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Date

From Page No. 49 Results:

Mch A 1026

| <u>RNA</u> | <u>% Uyb</u> | <u>Neg Ratio</u> | <u>Cpm</u> | |
|--------------------|--------------|------------------|---------------|----------------|
| Meb Meb | 53.7 > 53.6 | 37.7 > 37.7 | 9372 > 9353 | Total Counts = |
| Che | 53.4 | 37.6 | 9333 | Feo |
| Che Nas | 1.7 > 1.5 | 1.2 > 1.1 | 294 > 255 | 17461 |
| Not-cw | 1.2 | 0.9 | 216 | 17463 > 17462 |
| Nas Rbr | 1.1 > 1.4 | 0.8 > 1.0 | 188 > 236 | |
| | 1.6 | 1.1 | 284 | |
| Not-cw | 1.6 > 1.6 | 1.1 > 1.1 | 272 > 267 | |
| | 1.5 | 1.1 | 262 | |
| Rbr | 1.2 > 1.3 | 0.9 > 1.0 | 215 > 230 | |
| | 1.4 | 1.0 | 244 | |
| LUC | 1.4 | | 276 > 248 | |
| | | | 221 | |
| Meb | 71.3 > 71.6 | 60.4 > 60.7 | 10985 > 11032 | Mch B 1634 |
| | 71.9 | 60.9 | 11079 | Total counts = |
| Che | 1.5 > 1.4 | 1.2 > 1.2 | 225 > 211 | 15380 > 15403 |
| | 1.3 | 1.1 | 196 | 15426 |
| Nas | 6.8 > 5.4 | 5.7 > 4.6 | 1042 > 827 | |
| | 4.0 | 3.4 | 612 | |
| Not-cw | 6.9 > 7.1 | 5.8 > 6.0 | 1060 > 1083 | |
| | 7.2 | 6.1 | 1105 | |
| Rbr | 3.8 > 5.0 | 3.2 > 4.2 | 575 > 765 | |
| | 6.2 | 5.2 | 955 | |
| LUC | 18 1.2 | | 184 > 182 | |
| | | | 180 | |

See Book 678 for Mch B 1638 Results
cont to 678.1

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